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Standard DNA amplification assay

Suggested Protocol using Taq-E Polymerase

This protocol serves as a guideline for primer extensions. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

Pipette the following into a PCR tube, mix and make up to a final volume of 50 μ L.

We recommend dispensing all reagents on ice, adding the enzyme last. It is important to vortex all buffers and MgCl₂ solutions before use to remove any gradients that may result from repeated freeze/thaw steps.

If you do have already your own PCR-Protocol established, please use your existing pipetting scheme and Thermocycler protocol.

Table 1: PCR reaction components

Components	Quantities
Template DNA	1 ng - 10 ng plasmid DNA or 5 ng - 500 ng genomic DNA
Nucleotides	1 μ L (10 mM) each of dATP, dCTP, dGTP, dTTP
10X amplification buffer	5 μ L
25 mM MgCl ₂	3.0 μ L (if no complete buffer is used)
primer 1:	4-7 μ L of 3 μ M solution (10 - 20 pmole absolute)
primer 2:	4-7 μ L of 3 μ M solution (10 - 20 pmole absolute)
sterile, bidistilled water	up to 50 μ L
Taq E-Polymerase	0.25 - 1.0 μ L (1.25 - 5 units)

Table 2: MgCl₂ concentration in a 50 μ L reaction (complete buffer)

Final MgCl ₂ conc. in reaction (mM)	2.5	3.0	3.5	4.0	4.5	5.0	5.5
Additional volume of 25 mM MgCl ₂ per reaction (μ L)	0	1	2	3	4	5	6

Table 3: MgCl₂ concentration in a 50 μ L reaction (incomplete buffer)

Final MgCl ₂ conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25 mM MgCl ₂ per reaction (μ L)	3	4	5	6	7	8	9

Notes:

- Drops should be collected by centrifugation and 50 μ L of mineral oil should be layered upon the reaction mixture.
- Program the thermal cycler according to the manufacturer's instructions. Each programme should start with an initial heat incubation step at 94°C for 3-5 minutes!
- Recommended elongation time is 1 minute per 1kb of target!
- For maximum yield and specificity, temperatures (annealing) and cycling times should be optimised for each new template target or primer pair.